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Signed Andrew Gersey

Dated 29 July 2003

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
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NP9 1RH

1. Your reference	REP07140GB	
2. Patent application number *(The Patent Office will fill in this part)*	0215878.0	
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	Smart Holograms Limited 112 Hills Road Cambridge CB2 1PH	
Patents ADP number *(if you know it)*	8421273001	
If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom	
4. Title of the invention	CELL DETECTION	
5. Name of your agent *(if you have one)*	Gill Jennings & Every	
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Broadgate House 7 Eldon Street London EC2M 7LH	
Patents ADP number *(if you know it)*	745002	

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	4 ✓
Claim*(s)*	1 ✓ CK
Abstract	
Drawing*(s)*	1 + 1

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	NO

11. For the applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date
9 July 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

R E Perry
020 7377 1377

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a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
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CELL DETECTION

Field of the Invention

This invention relates to the detection of cells, e.g. using a holographic sensor.

Background to the Invention

Rapid identification of cells, in particular pathogenic cells, is of vital importance in diagnostics and biodefence. Whilst there are a number of competing technologies available to aid in this process, such as ELISA and PCR, the definitive identification of a microbial pathogen is still a time-consuming, laboratory-based procedure.

ELISA kits for the detection of agents such as *Bacillus anthracis* are available. These kits are highly specific to the target organism, showing no cross-reaction with closely related *Bacillus* species. They are, however, somewhat insensitive, requiring in the order of 10,000 cells, in order to avoid false negatives; this quantity of cells is somewhat more than a human infective dose of a microbe such as *Bacillus anthracis.*

PCR technology provides a fast, accurate and rapid means for determining the identity of a disease-causing agent. Unfortunately, this technology is sensitive to environmental contamination, meaning that sample pre-treatment is necessary in many instances. This technology is also expensive and requires highly trained personnel.

Neither of these methods is readily compatible with conventional microbiology techniques. While they may be used in some circumstances to determine the identity of a microbe in a large or pure sample, they do not readily lend themselves to direct comparison with laboratory assays in which cells are cultured and identified using classical microbiological methodologies. Nor do they provide a means for capturing viable cells for definitive identification.

Holographic sensors may be used for the detection of a variety of analytes. WO-A-9526499 discloses a holographic sensor, based on a volume hologram. This sensor comprises an analyte-sensitive matrix having an optical transducing structure disposed throughout its volume. Because of this physical arrangement of the transducer, the optical signal generated by the sensor is very

sensitive to volume changes or structural rearrangements taking place in the analyte-sensitive matrix as a result of interaction or reaction with the analyte.

Summary of the Invention

According to an aspect of the invention, a method for the detection of a cell comprises immobilising the cell in a device also containing an optical sensor, and introducing a growth medium. The sensor is sensitive to a product of the cell's growth, and a change in an optical characteristic of the sensor is detected. Preferably, the cell is immobilised using an antibody.

According to another aspect of the invention, a device for use in a method of cell detection comprises an antibody, an optical sensor and inlets for a sample and for a growth medium. The antibody is or can be immobilised in the chamber or elsewhere in the device that provides a fluidic link with the sensor. The device preferably comprises a container comprising a buffer solution and an outlet leading to the sample inlet of the chamber. The antibody may be immobilised on a wall of the chamber, or on a magnetic particle.

The invention allows rapid, accurate identification of the target organism, with the specificity of ELISA technology. Detection can be made under a wide range of conditions, e.g. at sub-infectious concentrations. A device of the invention may be simple to operate and compatible with standard laboratory techniques. By directly interfacing a device of the invention with PCR technology, full integration with laboratory-based diagnostics is possible.

Description of Preferred Embodiments

A cell may be captured with an agent, such as an antibody. The cell is then cultured *in situ*, in a range of determinative microbiological growth media and in the presence of the holographic sensor. Products released into the growth media during germination may also be detected. Germination of bacterial spores, as well as subsequent growth, typically requires the presence of specific nutrients, divalent ions and a specific pH range. The requirements for germination may differ from those for outgrowth.

Upon capture, detection can be made by monitoring the metabolic activity of the cell. The sensor is "optical" in the sense that it can be observed using optics. Typically, it is a holographic sensor. A holographic sensor can be used to detect species such as biodegradative enzymes or very small changes in pH

and redox potential. For example, acidic species can be detected using a pH-sensitive holographic element. As the pH changes, the holographic element undergoes a swelling or contraction, resulting in a colour change of the reflected wavelength. The sensor that is used may be as described in WO-A-9526499 or WO-A-9963408, the contents of which are incorporated herein by reference.

A method of the invention can be used to detect pathogens of bio-warfare and bio-terrorist interest (e.g. *Yersinia pestis* and *Francisella tularensis*) as well as pathogens of interest in environmental and medical monitoring (e.g. *Legionella* spp. and *Salmonella* spp.). Other bacteria which may be detected include *Bacillus anthracis, Bacillus globigii*, *B. megaterium* and *B. subtilis.*

An example of whole cell detection is that of the bacterium, *Legionella pneumophilia*, which is associated with Legionnaire's disease (Legionellosis) and Pontiac fever. *L. pneumophilia* serogroup1 is the most frequently implicated in human disease and is usually found in aqueous environments. The bacteria survive in low numbers in routine water treatment and reproduce to high numbers in warm, stagnant water. The bacterium may be immobilised with an appropriate monoclonal antibody. For example, a purified IgG3 class mouse monoclonal antibody that recognises the lipopolysaccharide antigen of heat-resistant *L. pneumophilia* serogroup 1 is commercially available.

The immobilised cell is then cultured, and a metabolic product detected. One approach is to use a pH-sensitive hologram; *L. pneumophilia* hydrolyses hippuric acid generating benzoic acid, producing a swelling and colour change of the hologram. A similar approach can be used to detect the ability of the organism to hydrolyse penicillins. Any additional penicillin will be hydrolysed by the intrinsic β-lactamase of *L. pneumophilia*, and the resulting penicilloic acid may be detected using a pH-sensitive hologram. An alternative approach exploits the fact that *L. pneumophilia* has endogenous oxidase activity, generating hydrogen peroxide from appropriate substrates. Hydrogen peroxide reacts with iodine to generate iodide ions. In the presence of iodine, a holographic sensor comprising silver grains can be used to detect hydrogen peroxide since any iodide ions formed react with silver to form silver iodide. Holograms can respond to added and enzymatically generated hydrogen peroxide *via* this mechanism.

A device of the invention comprises an inlet (such as a flip-top well) into which a test sample is placed. The sample is preferably transferred by a fluid (e.g. a buffer solution) to a growth chamber comprising the sensor and the immobilising agent, preferably an antibody, which captures the organism prior to the addition of growth medium. Antibodies may be immobilised on one or more walls of a chamber or on magnetic particles upstream of the growth chamber; if desired, the particles may be transferred to the chamber using a magnet present in the device. Alternatively, a cell may be immobilised upstream of the sensor, provided that the two have a fluidic link, i.e. that a product of the cell can flow into contact with the sensor. A growth medium is then fed into the device, and the growth of any specifically bound organisms can be detected, by observation of the sensor. A change of a property of the hologram can be observed using any suitable apparatus, e.g. as described in WO-A-9526499.

A device of the invention preferably comprises multiple cell capture chambers. The test sample may be mixed with a basal growth medium, which can be added to a series of fermentation wells, each containing dried carbon and/or nitrogen sources and a holographic sensor. Should magnetic particles be used, then each cell is preferably backed by a magnetic strip to capture the particles on which the test organism is immobilised. The device may further comprise a well downstream from the growth chamber, to collect excess and waste samples.

The following Example illustrates the invention.

Example

Bacillus subtilis was detected in microbial culture. A metabolic product of the bacterium is protease, which degrades a gelatin-based holographic sensor. As the gelatinous support medium degrades, it becomes increasingly spongy and expands.

Mid-exponential phase culture (in nutrient broth) was inoculated into a cuvette containing the hologram, and a reflection spectrometer used to measure the peak wavelength at 10 minute intervals over 15 hours at 30°C. A positive result for protease was shown by the peak wavelength undergoing a red-shift. Figure 1 shows the red-shift of the peak wavelength of reflection over the 15 hour period.

CLAIMS

1. A method for the detection of a cell, which comprises immobilising the cell in a device also containing a sensor, and introducing a growth medium, wherein the sensor is sensitive to a product of the cell's growth; and detecting any change in an optical characteristic of the sensor.

2. A method according to claim 1, wherein the cell is immobilised on a magnetic particle.

3. A method according to claim 1 or claim 2, wherein the cell is a spore cell.

4. A method according to any preceding claim, wherein the cell is a bacterial cell.

5. A method according to claim 4, wherein the bacterium is selected from *Bacillus anthracis*, *Bacillus globigii*, *Bacillus subtilis*, *Bacillus megaterium*, *Legionella pneumophilia*, *Francisella tularensis*, *Yersinia pestis* and *Salmonella* spp.

6. A method according to any preceding claim, wherein the cell is immobilised by means of an antibody.

7. A method according to any preceding claim, wherein the sensor is a holographic sensor.

8. A device suitable for use in a method according to claim 6, which comprises a chamber including a sensor, inlets for a sample and for a growth medium, and means for immobilising an antibody in the chamber or elsewhere in the device that provides a fluidic link with the sensor.

9. A device according to claim 8, wherein the antibody is immobilised on a wall of the chamber.

10. A device according to claim 8, which additionally comprises the antibody immobilised on a magnetic particle, and the said means can provide a magnetic field.

11. A device according to any of claims 8 to 10, further comprising a container including a buffer solution, in connection with the sample inlet.

12. A device according to any of claims 8 to 11, which comprises a series of said chambers.

13. A device according to any of claims 8 to 12, wherein the sensor is a holographic sensor.

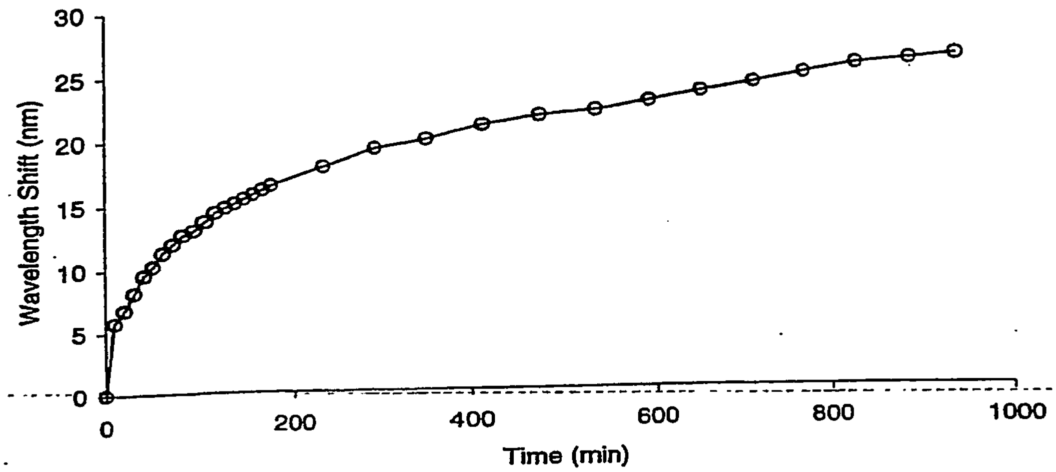

Figure 1 : Peak wavelength of gelatine hologram cultured with *B. subtilis*

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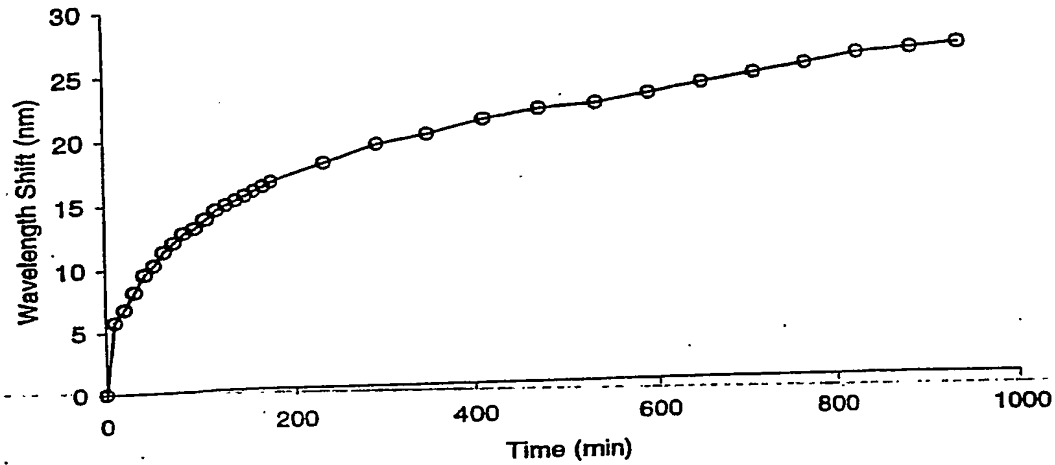

Figure 1: Peak wavelength of gelatine hologram cultured with *B. subtilis*

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